

Now remelt the material on the glass slide as before ; and when sufficiently fluidified to present a uniformly level surface (but *not burnt*, for this would destroy the tenacity of the cement and thus give it a crispness which, by cracking, would defeat all attempts at further reduction), quickly transfer the warmed slice (which should now be close at hand) to it, while with a little pressure the "*smoothed*" surface is brought into direct contact with that of the glass. Thus let it remain on the table where this is done until the glass feels cold to the touch.

After this reduce the slice to the thinness of a wafer over a very fine vertical rotating grinding-stone, or on a copper plate with emery powder and water, horizontally.

Now wash it well in water, and, placing the slide on a piece of buckskin leather spread on the table or over a level surface (to keep it from slipping) with the slice uppermost, continue the reduction in water with a piece of very fine siliceous limestone, that may be obtained from a statuary, of a convenient form (that is, one which will admit of the surface of the slice coming into direct and continuous contact with that of the limestone), with which it should be horizontally rubbed until reduced to the required thinness, which must be ascertained by repeatedly transferring the slice to the field of the microscope with an inch object-glass and high ocular. The nearer this thinness is approached the oftener this transfer should be made, washing the slice by dipping the slide into a bowl of water each time that it is examined.

When sufficiently reduced, wash the slide as before, and stand it up to drain until the slice is perfectly dry. Then cover with benzol, followed by balsam and thin glass, for preservation and more deliberate examination.

I make no apology for introducing these remarks, as the "process," although open to criticism and improvement no doubt, answers the purpose; and while inexperienced I myself should have been very glad of such aid. Dr. Holl suggested to me the use of shellac, which is the most valuable hint that I have received.

IV.—*On the Presence of Starch-granules in the Ovum of the Marine Sponges, and on the Ovigerous Layer of Suberites domuncula, Nardo.* By H. J. CARTER, F.R.S. &c.

It was but a short time since that I pointed out another instance of a structure like that of the cellular tissue of plants,

situated around the statoblast of freshwater sponges ('Annals,' 1882, vol. x. p. 367); the presence of starch-granules had been described in 1856, although not figured until 1859; and now I have to announce the latter also in the ovum of the marine sponges.

As this was brought to my notice by cutting up a fragment of the ovigerous layer of *Suberites domuncula* into small pieces, when, by pressure, both oil-globules and starch-granules made their appearance among the granular contents of the ova, I will particularly describe this layer before comparing these ova with those of other sponges.

Familiar to naturalists as *Suberites domuncula*, Nardo, ap. Schmidt, 1862 (Spong. Adriatisch.), = *Hymeniacidon suberea*, Bowerbank, 1866 (Mon. Brit. Spong. vol. ii. p. 200), has been for many years past, viz. from Aldrovandus, at the beginning of the 17th century (Johnston, Brit. Spong. p. 142), down to the present time, although not recognized as a *sponge* until Col. Montagu described it as such from specimens dredged on the coast of Devonshire circa 1812 (Werner. Mem. Edin. 1818, vol. ii. p. 100), no one appears to have noticed the striking manner in which its ova are deposited in a layer on that part of the hard object (generally a dead shell) over which it may have grown.

On the 6th January 1870, after a storm, I picked up on this beach (Budleigh-Salterton, South Devon) two specimens, and on the 4th September 1877 upwards of 150 were brought to me from the dredgings of a "trawler" about 20 miles off this shore, all of which had grown on dead shells of a similar kind, viz. *Turritella* and small *Buccinum*, tenanted either by a hermit crab (*Pagurus*) or annelid. All, or as many as I have examined, present the same kind of ovigerous layer, in which all the ova are in the same stage of development; so that we may infer, from the dates above mentioned and what will be stated hereafter, that throughout the year these sponges, if containing a dead shell or any similarly hard object, will have upon it a similar ovigerous layer.

Like most of its kind, too, *Suberites domuncula* not only grows over the dead shell, but as it grows encloses a large quantity of the fine detritus of the sea-bed in which it may have lived; so that it is impossible to free the smallest portion from this foreign material, and it is on this account equally impossible to obtain a satisfactory view of the finer elements of which the sarcodic substance of the sponge itself is composed.

Each specimen appears to be but a single individual ("person," Hæckel), as each has only a single ragged vent

situated on the most pendent or prominent part, which is the outlet of a well-developed excretory canal-system, whose branches pervade the extremely fine and compact structure of which the sponge is otherwise composed. The pinlike spicules &c. and the ovigerous layer that I am about to describe are also particularly evident; but as the sponge generally has been heretofore repeatedly noticed with the exception of this layer, I shall henceforth confine myself to a description of the latter only.

If a vertical section of one of these specimens be made, so that the incision may fall perpendicularly and longitudinally on the shell over which the sponge may have grown, and the two portions forcibly separated from each other, so as to expose the shell beneath (say a *Turritella* about $1\frac{1}{2}$ inch long), a yellowish chitinous layer composed of ova closely packed together (not unlike the nidamental layer of a mollusk) will be left upon the shell, corresponding in extent to the amount of the shell covered by the sponge, whether this be a part or the whole, and adhering so strongly that the whole of the sponge-substance may be washed off with a brush without disturbing the attachment (fig. 3). On the other hand, supposing that the sponge, as is commonly the case, be attached to the whole length of the *Turritella* and the specimen (having been preserved in a wet state) is put into dilute nitric acid, the shell part will be entirely dissolved away, leaving the ovigerous layer in this instance attached to the sponge, when a similar section with a sharp thin knife may be made to pass through both the sponge and the ovigerous layer together; and thus, by examining the object in water under a microscope, the thickness and structure of the latter may be easily ascertained.

The ovigerous layer may then be observed to be composed of a yellowish tough chitinous stratum of ova in juxtaposition, but only one ovum deep or thick (fig. 2), of which the part that was in contact with the shell is flat, thin, and even (fig. 2, *b*), but that towards the sponge thick and granulated by the convexities of the layer of ovarian capsules, which, from being compressed together, vary slightly in height, size, and shape, so that, although generally the stratum or ovigerous layer is only one 200th of an inch thick, the thickness towards the sponge is so far rendered irregular (fig. 2, *a*), while the horizontal diameter of the ova when viewed in a flat position on the sponge side is found to vary from the 180th to the 90th of an inch (fig. 3); hence the ovum, being flat below, convex above, and rendered more or less polygonal laterally by horizontal compression, fails to

present that spherical form which it would do if, as in other cases, it had been isolated in the midst of the sponge-tissue.

Taking the elements of the ovum one by one from without inwards, it will be found to consist of a thick chitinous capsule followed by a delicate membrane filled with the yelk. The capsule, as before stated, is convex towards the sponge, flat towards the shell, and polygonal laterally, presenting on its convexity a great number of minute circular granules or points arranged more or less hexagonally, more or less projecting beyond the surface, often possessing a punctum in the centre, and always connected with each other by a fold of the surface, so that it presents under high microscopic power a reticulated appearance (figs. 3, *a*, and 4). This part of the capsule is very thick when compared with the side towards the shell, being composed of five or more chitinous layers, amounting in all to 1-3000th inch, while the flat side hardly amounts to more than one of these layers. Interiorly it is smooth, where it is in contact with the delicate membrane investing the yelk, and appears to be uniformly closed on all sides; so that no aperture whatever could be detected in it.

The yelk, on the other hand, surrounded by this "delicate membrane," which is hardly demonstrable from its transparency and thinness, consists of spherical refractive granules about 1-12000th inch in diameter, among which are a great many oil-globules and starch-granules, the latter of a greyish-white colour, more or less oval in form, flat, and presenting a crack-like translucency in the centre, varying in size under 1-600th inch in diameter and becoming of the usual opaque blue colour under the influence of iodine, when they strikingly contrast with the whiteness of the yelk-granules generally, which do not become so coloured.

Such is the composition of this ovigerous layer, and such the characters of the contents of the ova in every instance that I have examined; so that it may fairly be assumed that under these circumstances none ever get beyond the granulation of the yelk in this position, whatever they may do afterwards when impregnation and the duplicative subdivision of the yelk destroys the individuality of the yelk-granules and leads on to the development of the embryo—a state which I have not witnessed, nor do I know where to find; but as the ovigerous layer is only to be seen over the surface of the hard objects enclosed by the Suberite so long as they exist, and no trace of such a layer or any ova can be discovered after the shell has disappeared, even when its *mould* still remains empty, which is often the case (as proved by a specimen where the

upper half of the *Turritella* on which the sponge had grown remains with the ovigerous layer still on it, while the lower part has disappeared and left not a trace of ova or the ovigerous layer behind, where both originally existed (fig. 1), and in another, where the original shell has passed away and a smaller one has been enclosed, apparently for the purpose of receiving a new ovigerous layer), it seems not impossible that impregnation and the further development of the ovum may take place with the disappearance of the shell, when the embryo at least could easily escape through that part of the capsule which was in contact with it, and which I have stated to be so extremely thin (fig. 2, *b*).

Let us now turn our attention for a few moments to the ova of other marine sponges, to see how far they agree with those of *Suberites domuncula*; and selecting *Isodictya simulans*, Bk., for this purpose out of several ova-bearing ones that I have, preserved in spirit, the ova will be found to be congregated in the sponge towards the base or oldest part, but not attached to the rock on which the sponge may have grown, as in *Suberites domuncula*. While thus isolated they present a spherical form so long as the yelk has not passed into the fully developed embryo; but when this is completed the embryo bursts through the delicate capsule which enclosed it, and then assumes the form of a conical shot or elongated cone with a little tuft at the obtuse end (the long cilia). Under the former or spherical condition, in my specimen, the grumous or clotted state of the contents and presence of spicules already show that the originally granular state of the yelk has been transformed through duplicate subdivision with its consequences, into the coming embryo, and thus the presence of starch, although evident under the use of iodine, is *now* very trifling, showing that in the egg of the Suberite, as in the seed of plants, it is abundant at the commencement and disappears in the usual way under germination.

The same observations apply to the ova of *Halisarca lobularis*, *Aplysina corneostellata* (*Darwinella aurea*), *Esperia*, and *Halichondria sanguinea*, Johnst., so that we here see the *animal* nature evinced in spite of the resemblance of the ova of sponges to the seed of plants; for while the sponge-embryo develops a root for fixation only, and a superstructure for supporting organisms that take in crude material for food, that of plants develops a root for nutrition as well as fixation and a leafy superstructure, viz. the "plumule," which grows by *endosmosis* through the spongioles at the ends of the radicles.

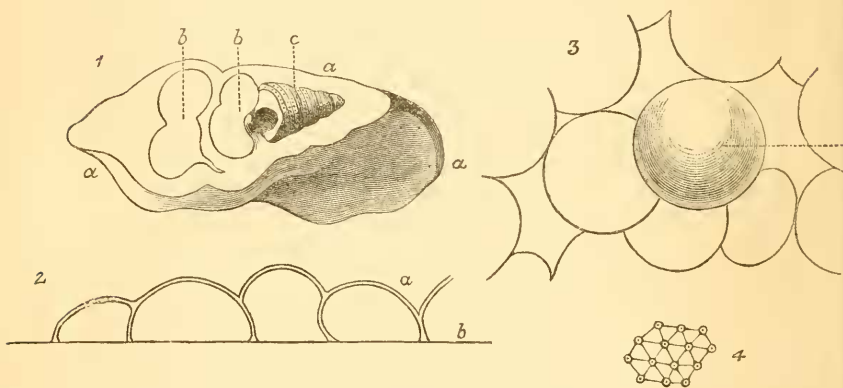
Having already gone into the subject of impregnation in

the Sponges ('Annals,' 1882, vol. x. p. 364), I have only to add here that, accompanying the ova of *Aplysina corneostellata* especially, which are about 1-120th inch in diameter, there are in my specimen many smaller spherical bodies averaging only 1-360th inch, which in size do not appear to be a second set of ova, although when dyed with aniline red they all (both ova and these bodies) become equally tinged and much more so than the other parts; but being respectively surrounded by a capsule formed of granuliferous cells, it is almost impossible to say what appearance their respective contents otherwise present. I think I can see the same also among the ova of *Isodictya simulans*, where the granulated appearance of *their* contents is not obscured by a capsule of this kind; and certainly in a thin slice of *Grantia compressa*, whose spongozoa in the living state were successfully fed with indigo, so that all the other elements (viz. larva, ova, and these granuliferous cells) remain *uncoloured*, while the blue spongozoa mark out the ampullaceous sacs with a sharpness which prevents all confusion; but whether these granuliferous bodies were spermatic or not I am unable to decide. It may not be unworthy of remark here, that while examining the freshwater sponges of Bombay, in 1856, I noticed granuliferous cells which seemed to me to be spermatic, and thus described them "provisionally" under the head of "spermatozoids" ('Annals,' 1856, vol. xviii. p. 227).

Lastly, another instance of the presence of spermatozoa in sponges has lately been added by Dr. N. Poléjaeff, of the University of Gratz, which was obtained from an examination of *Sycandra raphanus*, Häckel, in the *living* state, by making very thin slices and examining them under a high microscopic power, afterwards followed by the application of osmic acid and alum-carmin, to render the tail or cilium more evident ("Ueber das Sperma und die Spermatogenese bei *Sycandra raphanus*, Häckel," Separat-Abdruck aus dem lxxxvi. Bande der Sitzb. der k. Akad. Wissensch. 1 Abth. Nov. Heft, Jahrg. 1882; read 16th Nov. 1882). But still there is no character given to the spermatic cell, as I have before noticed, by which it may be satisfactorily recognized by the inexperienced student, if, indeed, there be any such; and, so far as the *granulation* goes, it is as conjectural to me now as it was in 1856.

Allusion is made (p. 4) to my also conjectural figures of the spermatozoon in *Grantia compressa* ('Annals,' vol. xiv. p. 108, pl. x. figs. 21-23), which are so much more like the monociliated cells of the larva at a very early period than the spermatozoa of *Sycandra raphanus* represented by Dr. Poléjaeff, that I am now more inclined than ever to regard them as such.

The ova of sponges in colour generally follow that of the parent sponges themselves, especially towards maturity, when this becomes more intense, and thus they contrast strongly with the rest of the substance. The ovabearing specimens which I possess are :—*Halisarca lobularis*, obtained from this shore in July 1874; *Aplysina corneostellata*, from Vigo Bay, by Saville Kent, F.L.S. &c., June 1870; *Isodictya simulans*, from this shore (here the ovum is white), July 1874 (at this time also I found *Esperia* and *Halichondria sanguinea* in an ovabearing state, but did not preserve any of them); lastly, *Grantia compressa*, from this shore, May 1871, viz. those individuals which were successfully fed with indigo. Dr. Poléjaeff does not give the date of his observing the spermatozoa in *Sycandra raphanus*, although, from his paper having been read in the month of November, it may be inferred that this took place during the preceding summer. It is desirable to add the dates of such observations, because they may not only be a guide to others, but finally fix the period of this mode of reproduction in the species.



EXPLANATION OF THE WOODCUTS.

- Fig. 1.* *Suberites domuncula*, Nardo, natural size. Section of, showing :—*a a*, sponge; *b b*, cavities left by the lower whorls of the enclosed shell (*Turritella*), which have disappeared; *c*, remaining portion of the shell, covered with the ovigerous layer.
- Fig. 2.* The same. Fragment of vertical section of ovigerous layer, showing :—*a*, convexities of capsules towards the sponge; *b*, flat membrane covering the shell. Scale about 1-24th to 1-1800th inch.
- Fig. 3.* The same. Fragment of ovigerous layer viewed from the sponge side, showing the juxtaposition of the ova : *a*, ovum, on which the granulation of the surface is depicted. Same scale.
- Fig. 4.* The same. Fragment of the granulated surface, much magnified. Scale about 1-24th to 1-6000th inch.